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REVERSED-PHASE LIQUID CHROMATOGRAPHIC RETENTION BEHAVIOUR OF DIMETHYLPHENANTHRENE ISOMERS

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SUMMARY

The retention behaviour in reversed-phase liquid chromatography of all 25 dimethylphenanthrene (DMP) isomers on a polymeric C₁₈ column was investigated and could be related to their molecular shape (length-to-breadth ratios and distortion angles of the aromatic systems). These two descriptors of the aromatic molecular shape were generated by a molecular mechanics program and will be useful in the prediction of behaviour when reference standards are not available. An application of this concept to the identification of DMP isomers in a crude oil sample is presented.

INTRODUCTION

Complex mixtures of alkylated polycyclic aromatic hydrocarbons (PAHs) present in natural matrices contain numerous isomeric structures. Increasing attention has been paid to the separation and the quantification of individual alkylated PAHs both in organic geochemistry (alkylated PAH patterns are typical of the maturation of the sedimentary organic matter^{1–3}) and in environmental chemistry (isomeric forms often have different carcinogenic or mutagenic activities).

One of the most commonly used techniques for the separation of PAHs is high-performance liquid chromatography (HPLC). Particularly reversed-phase liquid

chromatography (RPLC) on chemically bonded C₁₈ stationary phases has been successfully applied to the separation of methyl-substituted PAH isomers⁴⁻⁹. Recent studies have demonstrated enhanced selectivity of polymeric C₁₈ phases compared with conventional and widely used monomeric C₁₈ phases^{7,8,10}. Moreover, Wise *et al.*⁵ observed a relationship between the molecular shape of a PAH solute, defined as the length-to-breadth ratio (L/B), and its reversed-phase LC retention. Particularly when applied to three planar dimethylphenanthrene (DMP) isomers, the relationship correctly predicted their elution order on a monomeric and a polymeric C₁₈ column⁵.

In this study, the retention behaviour of all 25 DMPs in RPLC on a polymeric C₁₈ phase was investigated. Special emphasis was placed on the effect of aromatic non-planarity described by the dihedral angle between the aromatic rings. A molecular mechanics method applied to each PAH molecule was used for the optimization of the geometry and the calculation of L/B values.

EXPERIMENTAL

Materials

The DMP and ethylphenanthrene (EtP) isomers listed in Table I were synthesized at the Institute of Petroleum and Organic Geochemistry, Jülich, F.R.G. The DMP and EtP isomers were prepared from dimethylstilbenes by photocyclization and dehydrogenation³. Some DMPs obtained as mixtures were separated by HPLC. Identification of DMP isomers was based on ¹H NMR data. The purity of the standards determined by capillary gas chromatography was generally greater than 97%.

The crude oil sample came from an Indonesian oil field. Sample preparation and PAH extraction have been described elsewhere².

Chromatography

Chromatographic separations were performed on columns (25 cm × 4.6 mm I.D.) containing 5- μ m particle size Supelcosil LC-PAH polymeric C₁₈ stationary phase (Supelco, Belfonte, PA, U.S.A.), equipped with guard columns (3 cm × 4.6 mm I.D.). Two LC systems were used. The first operated at room temperature and consisted of two LDC pumps (Constametric II and III), an LDC computerized solvent programmer (CCM), a Waters Assoc. injector (Wisp 710B) and an LDC Spectromonitor II UV-absorption detector. The second system operated at 35°C and was used only for the determination of the retention indices, and included a Spectra-Physics (Darmstadt, F.R.G.) pump, a Beckman (Berkeley, CA, U.S.A.) Model 210 injection valve, a Knauer (Berlin, F.R.G.) oven and a Knauer variable-wavelength detector. Acetonitrile-water (50:50, v/v) was used as the mobile phase at a flow-rate of 2 ml min⁻¹. The UV absorption was monitored at 254 nm.

The LC retention data were reported as log I , where I is the retention index as described by Popl *et al.*¹¹:

$$\log I_x = \log I_n + \frac{\log R_x - \log R_n}{\log R_{n+1} - \log R_n}$$

where I_x is the retention index of compound x , I_n the retention index of the

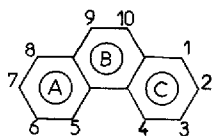


Fig. 1. Numbering of the phenanthrene molecule.

standard compound which elutes just prior to x , R_x the retention time of x , R_n the retention time of the standard eluting before x and R_{n+1} is retention time of the standard eluting after x . All retention times are corrected retention times (retention time of the solute minus that of the presumably unretained acetone)¹². The following values ($\log I$) were assigned to the PAH standards used for calibration: phenanthrene 3, benz[*a*]anthracene 4 and benzo[*b*]chrysene 5^{7,11}. The $\log I$ values listed in Table I are mean values of two analyses.

Length-to-breadth ratios (L/B) and PAH non-planarity

The calculations were carried out for the DMP isomers with a VAX computer (installed at the CRPP, University of Bordeaux I) using the MM2MP2 empirical force field method¹³. The calculations yielded the geometry of the molecule at the energy minimum and also the dihedral angles between aromatic rings A and C (see Fig. 1). The L/B values were calculated based on the design of a rectangle fitted to the PAH molecule. Calculations for EtP isomers were carried out roughly without using the MM2MP2 program.

RESULTS AND DISCUSSION

A chromatogram of DMP standards used in the determination of retention indices is shown in Fig. 2.

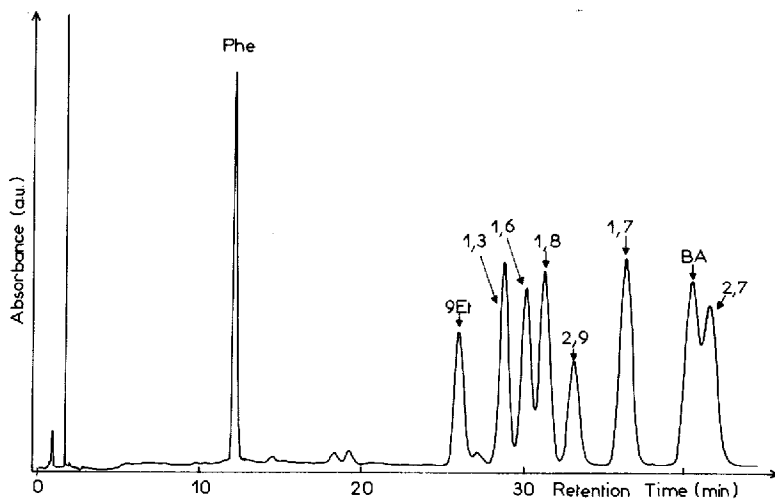


Fig. 2. Partial reversed-phase chromatogram of reference DMP standards used in the determination of LC retention indices (see text for experimental conditions). Phe = phenanthrene; 9-Et = 9-ethylphenanthrene; 1,3 = 1,3-dimethylphenanthrene, etc.; BA = benz[*a*]anthracene; benzo[*b*]chrysene (retention time 270.4 min) not shown.

TABLE I

RETENTION INDICES AND SHAPE PARAMETERS FOR DIMETHYL- AND ETHYLPHENANTHRENE ISOMERS

Log I values were determined by experiments whereas log I_c values were calculated through the correlation equation (see text).

Compound	Log I	Log I_c	Deviation (%)	L/B	Dihedral angle ($^\circ$) ^a	Length (Å)	Breadth (Å)
1,2-DMP	3.80	3.85	+1.3	1.58	0.10	12.60	7.95
1,3-DMP	3.72	3.70	-0.5	1.28	0.05	11.80	9.20
1,4-DMP	3.67	3.73	+1.6	1.32	6.95	11.80	8.95
1,5-DMP	3.67	3.72	+1.3	1.31	6.40	11.80	9.00
1,6-DMP	3.76	3.75	-0.2	1.37	0.05	11.90	8.65
1,7-DMP	3.91	3.86	-1.2	1.59	0.05	12.60	7.90
1,8-DMP	3.79	3.84	+1.3	1.55	0.00	11.85	7.65
1,9-DMP	3.68	3.71	+0.8	1.28	0.05	11.80	9.20
1,10-DMP	3.65	3.71	+1.6	1.29	0.45	11.90	9.20
2,3-DMP	3.72	3.76	+1.1	1.38	0.00	12.70	9.20
2,4-DMP	3.75	3.76	-0.2	1.38	3.35	12.70	9.25
2,5-DMP	3.76	3.76	0.0	1.38	3.10	12.70	9.20
2,6-DMP	3.83	3.76	-1.6	1.40	0.00	12.45	8.90
2,7-DMP	4.01	3.91	-2.5	1.70	0.00	13.45	7.90
2,9-DMP	3.84	3.75	-2.3	1.37	0.00	12.60	9.20
2,10-DMP	3.78	3.74	-0.8	1.37	0.00	12.60	9.20
3,4-DMP	3.63	3.72	+2.5	1.31	11.90	11.95	9.10
3,5-DMP	3.67	3.71	+1.1	1.29	3.90	11.90	9.20
3,6-DMP	3.69	3.73	+1.1	1.33	3.73	11.85	8.90
3,9-DMP	3.75	3.63	-3.2	1.12	0.00	11.70	10.45
3,10-DMP	3.71	3.63	-2.1	1.12	0.00	11.70	10.45
4,5-DMP	3.42	3.71	+8.5	1.29	27.20	11.70	9.10
4,9-DMP	3.65	3.63	-0.5	1.29	5.70	11.80	10.45
4,10-DMP	3.64	3.63	-0.2	1.13	6.50	11.80	11.45
9,10-DMP	3.64	3.70	+1.6	1.28	1.28	11.70	9.15
1-EtP	3.70	n.d. ^b	n.d.	1.59	n.d.	12.64	7.95
2-EtP	3.78	n.d.	n.d.	1.77	n.d.	14.06	7.95
3-EtP	3.65	n.d.	n.d.	1.09	n.d.	10.69	9.81
4-EtP	3.55	n.d.	n.d.	1.09	n.d.	11.60	10.64
9-EtP	3.65	n.d.	n.d.	1.09	n.d.	11.60	10.64

^a Angles between rings A and C (see Fig. 1).

^b n.d. = Not determined.

The DMP isomers differ significantly in L/B values and also in dihedral angles (see Table I). DMP molecules with a methyl group in the bay region (position 4 and 5) exhibit strained molecular structures with large dihedral angles (more than 27° for 4,5-DMP and about 12° for 3,4-DMP). The theoretical calculations are in good agreement with X-ray crystallographic measurements¹⁴⁻¹⁶. The compounds with large dihedral angles generally elute before the unstrained DMP with low L/B and log I values. On the other hand, unstrained long or narrow DMP molecules are more strongly retained than those having a square-like shape. For example, 2,7- and 1,7-DMP exhibit high L/B and log I values.

The "slot model" previously proposed by Sander and Wise⁴ mimics schematic-

ally the insertion of PAH molecules into the stationary phase. Planar molecules will be able to fit more easily than non-planar molecules into the slots of the bonded phase. The latter molecules interact weakly with the stationary phase and will be retained less. Differences in retention can be attributed to the thicknesses of the non-planar solute molecules, which are well described by the dihedral angles (D); the interaction of methyl groups will twist the aromatic molecule in certain cases instances, which would hinder penetration of the solute into the slots.

Some interesting features could be drawn from Fig. 3, which shows the correlation between LC retention ($\log I$) and the L/B values for the 25 DMP isomers. The correlation equation obtained for 24 DMP (4,5-DMP was omitted) is $\log I_c = 0.366L/B + 3.234$ with a correlation coefficient $r = 0.713$. Predicted $\log I$ values were calculated and compared with the measured values. The average deviation was less than 2%, with high values for highly strained compounds (4,5-DMP and 3,4-DMP).

The correlation coefficient (r) between retention index ($\log I$) and L/B values is 0.67 for the complete set of DMPs, increases to 0.79 with 23 DMPs when 3,4- and 4,5-DMP are neglected and reaches 0.85 with a set of 14 DMPs when all the compounds with dihedral angles greater than 3° are neglected. A correlation coefficient of 0.73 is obtained when retention indices are plotted as a function of the molecular length (L) and 0.46 for molecular breadth (B) (Table I). All this information indicates that (a) the retention behaviour of DMP isomers is mainly controlled by the length of the molecules and (b) when plotted against L/B , negative deviations of $\log I$ from the average linear trend are due to non-planarity of the molecules resulting from steric crowding of the methyl groups.

Most of the non-planar compounds with dihedral angles greater than 3° are eluted before the planar compounds (Fig. 3). Only 2,4- and the 2,5-DMP, despite having dihedral angles of 3.35° and 3.10° , respectively, are eluted together with the others DMP having average $\log I$ values. In this instance, the steric hindrance of the methyl group in position 4 or 5, which tends to reduce the retention of the molecule, is compensated for by the effect of the methyl group in position 2. This group has its greatest extension in the direction of the long axis of the molecule, and thus tends to increase the retention. Most of the DMP isomers bearing at least one methyl group in

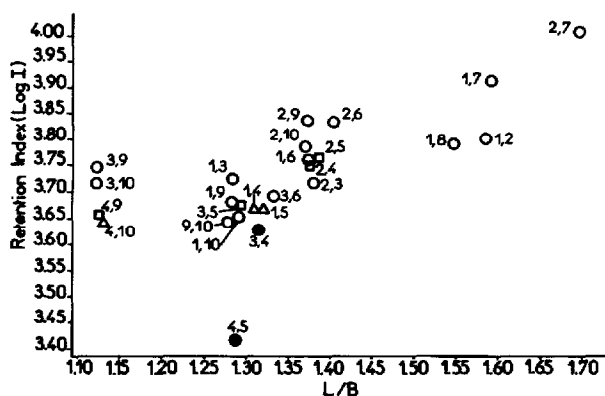


Fig. 3. Plot of LC retention indices versus L/B for all 25 DMP isomers with dihedral angles between 0° and 27.2° ($\circ = 0-3^\circ$; $\square = 3-6^\circ$; $\triangle = 6-10^\circ$; $\bullet = >10^\circ$).

position 2 or 7 also exhibit high retention indices and high L/B values. The methyl group in this instance is in the same direction as the long axis of the molecule and tends to increase the molecular length (L) and consequently the retention on the stationary phase.

Analytical applications

Fig. 4 shows the partial HPLC trace obtained for the triaromatic fraction of a crude oil. True identifications of the DMP isomers were achieved by capillary gas chromatography–mass spectrometry and high-resolution Shpol'skii spectroscopy^{3,17}. Despite the great selectivity of the column, some DMPs, are co-eluted as expected owing to their similar $\log I$ values. A difference of 0.03 $\log I$ units between isomeric compounds allows the separation of DMP isomers in the collected chromatographic fractions without any significant interferences. The non-planar DMPs and EtPs with strained structures (methyl groups in position 4 or 5, ethyl group in position 4) were not present in the natural sample studied, as reported in previous investigations for other sterically hindered aromatic compounds^{2,3,6}. Such trends demonstrate the importance of theoretical information obtained by calculations for the prediction of retention behaviour and the presence of alkylated PAHs in natural samples.

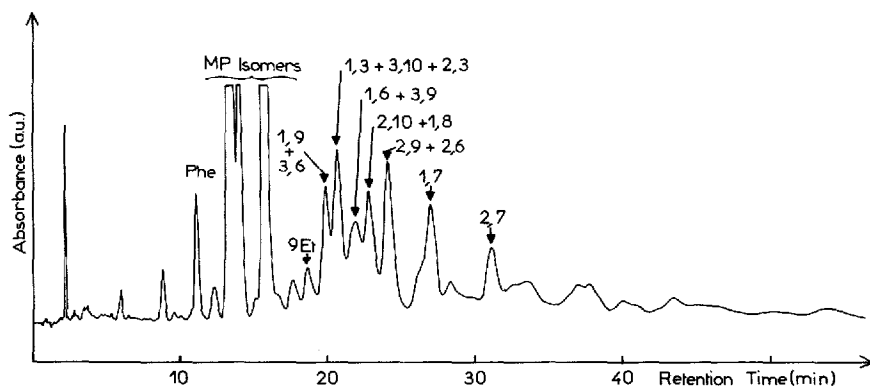


Fig. 4. Reversed-phase chromatogram of a triaromatic fraction from a crude oil. Identification of individual DMP isomers was done on collected fractions analysed by high-resolution Shpol'skii spectroscopy (see ref. 3 and text for experimental conditions). Phe = phenanthrene; MP = methylphenanthrenes; 9Et = 9-ethylphenanthrene; 1,9 = 1,9-dimethylphenanthrene, etc.

CONCLUSIONS

This study illustrates that the length-to-breadth ratio and the thickness described by dihedral angles provide a measure of the shape of aromatic isomers. These descriptors could well predict the elution order of DMPs on polymeric reversed-phase C_{18} stationary phases according to their molecular shape. The natural occurrence of specific DMP and EtP isomers could also be predicted according to their strained/non-strained conformation.

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